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INTERFERON INDUCING PROPERTIES OF CERTAIN STRAINS OF TICK-BORNE ENCEPHALITIS VIRUS

by

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13. ABSTRACT

The study of the correlation between the production of interferon and the multiplication of tick-borne encephalitis (TE) in mice in relation to the degree of pathogenicity of viruses, routes of their inoculation and the temperature of maintenance of inoculated animals has shown that a highly pathogenetic strain, S of M, of virus TE with any method of inoculation stimulated the production of a large quantity of the interferon in the brain of mice while, with the inoculation of a weakly pathogenetic strain, S of K, a large quantity of interferon is in the brain only with the intracerebrum inoculation of the virus. An increase of temperature of maintenance of the mice to 35° led to a certain acceleration of viral reproduction and the accumulation of interferon even though the maximum titres of that and the other fell off equal to the control and the test.

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INTERFERON-INDUCING PROPERTIES OF CLINICAL STRAINS OF TICK-BORNE ENCEPHALITIS VIRUS

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21 October 1963

The study of the correlation between the production of interferon and the multiplication of tick-borne encephalitis (TE) in mice in relation to the degree of pathogenicity of viruses, routes of their inoculation and the temperature of maintenance of inoculated animals has shown that a highly-pathogenetic strain, Sof A., of virus TE with any method of inoculation stimulated the production of a large quantity of the interferon in the brain of mice while, with the inoculation of a weakly-pathogenetic strain, Sof K, a large quantity of interferon is in the brain only with the intracerebral inoculation of the virus. An increase of temperature of maintenance of the mice to 35° led to a certain acceleration of viral reproduction and the accumulation of interferon even though the maximum titres of that and the other fell off equal to the control and the test.

The accumulation of interferon in various organs has been studied in many animals [2, 4, 5, 11, 13]. So, for example, Vinter [4], researching interferon in the various organs and serum of mice after inoculation by the virus of the Eastern Nile, discovered the highest concentration of interferon. Interferon was found as well in a high concentration in the serum of mice which had been inoculated with the virus of New Castle's disease.

With the aim of the present research, the study has been the production of interferon in the brain and serum of mice, which are infected with highly-pathogenetic and weakly-pathogenetic strains of virus TE, as well as the influence of the temperature of the maintenance of the mice in the process of the production of interferon. In addition, it is interesting to study the influence of exogenous interferon in the experimental viral infection, especially since the published data of the preventive role of interferon are sufficiently contradicted [2, 5, 7].

MATERIAL AND METHODS

Strain of E, isolated in 1937, went through many passages of the brain of white mice and were pathogenic for them with any route of inoculation.

Strain of E, weakly pathogenic for mice with the peripheral route of inoculation, was obtained from persistently infected cultures of cells Ver-2 by virus of E.

Virus-containing material was injected into the mice in the brain (0.03 ml), intravenously (1 ml), intraperitoneally (0.5 ml), through the mouth (0.25 ml) and hypodermically (0.25 ml). Through each day, beginning from the moment of inoculation up to death of the animals, blood was gathered from 15 mice of each group, and then brain samples were taken. Serum was obtained from the blood and a 10% suspension was prepared from the brain, part of which was utilized for the titration of the virus in cells CFEV, and from the remaining material interferon was prepared. For this test, we soured 5% portions with a solution of HCl up to pH 2.0 and kept it at 4° in flow for 24 hours, and then with a 6 n. solution of NaOH pH was brought up to 7.2-7.4. We subjected controlled experiments from the serum and brain of diseased mice to similar routes. We centrifuged the obtained preparation for 30 min. at 3,000 RPM, and we titrated it in the intertwined culture of cells L against 100 TCD$_{50}$ virus of encephalomyocarditis. We considered the results on the 3rd day, considering the titre of interferon the last of its cultivation, which preserves the culture from cytopathic activity of the virus encephalomyocarditis.

During the experiment of the preventive action of interferon of the cerebral interferon containing 1250 units/ml, we inoculated the mice in the abdominal cavity or intravenously with a quantity of 1 ml or 0.03 ml in the brain. Part of the mice received the same dose of interferon repeatedly throughout 24 hr.

Throughout 24 hours after the first inoculation or throughout 3 hours after the 2nd inoculation, we infected the mice intra-abdominally with 100 LD_{50} of a virus of the E strain of Rosettar. We inoculated the standard mice with such a dose. We noted the death of the mice daily. We utilized control mice and mice of the BALB line weighing 18-16 g. in the experiments.

RESULTS AND DISCUSSION

The experiments showed that with any route of infection with a highly-pathogenetic strain, Sof M of virus TE, in the brain of mice, a significant build up of interferons developed for which its greatest quantities (640-1280 units/ml) corresponded to the day of the maximum accumulation of virus in the brain--on the eve of the clinical displays of the disease and at the elevation of the clinic. After peripheral infection of the virus, we accordingly found interferon in the brain somewhat later than after their inoculation in the brain. So after hypodermic inoculation of virus Sof M, the maximum quantities of virus and interferon are displayed on the 9th day and with inoculation in the brains and intravenously, on the 5th day.

After inoculation with weakly-pathogenic strain Sof K under the skin, the titres of the virus in the brain of the infected animals were lower than after infection with virus Sof M. The titres of interferon were also lower. And so, the maximum titre of Sof K appeared on the 7th day and corresponded to 5.33 lg $TTSD_{50}$/ml, and the titre of interferon to 320 units/ml. However, with the inoculation of those viruses in the brain, the titres of the virus and interferon were sufficiently high: 7.0 lg $TTSD_{50}$/ml and 1280 units/ml.

In the serum, we periodically detected low titre of antivirus activity (8-16 units/ml); however, the serum of uninfected animals also irregularly displayed inhibiting activity to the virus of encephalomyocarditis. Therefore, the virus-inhibiting activity of the serum is difficult to relate to the production of interferon during the development of viral infection caused in the mice by virus TE.

By such a method, in the brain of the mice infected with virus TE, a large quantity of interferon can be detected since the virus breeds up to high titres there. A series of authors mention the connection between the reproduction of the viruses and the detection of interferon in the organisms of mice infected with virus TE [13], both Sindbis [14] and Koksaki A6 [1]. Information is available on the increase of production of interferon with higher temperatures of animal maintenance and on the influence on the flow of infection [10, 11].

In the second series of experiments, we studied the influence on the production of interferon of a rise (to 39°) and a decrease (to 4°) of the temperature of maintenance of the mice infected with virus TE. A lowering of temperature did not lead to a noticeable change of titres nor viruses nor interferon in comparison with the control. With a rise, some acceleration of the reproduction of viruses and accumulation of interferon was observed even though the maximal titres of that and the other remained equal in the control and in the experiment. So on the third day, the titre of virus Sof M in the control was 0.7 lg $TTSD_{50}$/ml and interferon was 40 units/ml; on the same line, at 39° the titre of the virus made up 5.33 lg $TTSD_{50}$/ml. On

the 5th day, the titre of the virus in control and in the experiment made up 5.5 lg TTD_{50}/ml and 1280 units/ml interferon. An analogous picture is observed in the experiments with virus Sof K (Table 2). By such a method, with the maintenance of the mice a low (4°) and high (35°) temperatures, the maximum litres of interferon in the brain corresponded to the day of the greatest accumulation of virus, as the mice maintained at a normal temperature.

Table 1

Titres of the virus (in lg $TTSD_{50}$/ml) and interferon (in units/ml) in the brain of mice infected with pathogenic and weakly-pathogenic strains of virus TE, according to strain and route of inoculation.

	Sof II							
	brain		intravenously		hypodermically		through the mouth	
	titre							
Day after infection	virus	inter-feron	virus	inter-feron	virus	inter-feron	virus	inter-feron
1st	1.5	0	1.67	0	1.0	0	0	0
2nd	3.5	0	3.33	0				
3rd	5.0	40	5.3	40	4.0	40	1.5	0
4th	7.0	340	7.0	340				
5th	7.7	1280	7.7	1280	5.0	320	3.5	40
6th								
7th					6.5	1280	5.5	340
8th								
9th					7.5	1280	7.0	340
10th								
11th					6.0	40	3.5	40

	Sof K					
	brain		intravenously		through the mouth	
	titre					
Day after infection	virus	inter-feron	virus	inter-feron	virus	inter-feron
1st	1.0	0	1.5	0	0	0
2nd						
3rd	3.0	0	3.5	80	1.0	0
4th						
5th	4.5	40	5.5	80	3.5	0
6th						
7th	5.33	1280	5.5	340	5.5	0
8th						
9th					4.0	0
10th						
11th					2.5	0

Probably, with TE, the endogenic interferon does not play an active role in the protection of animals from infection. Kirn and his co-authors [3] revealed a similar picture with mice infected with virus Sindbis and maintained at 35°.

Table 2

Titres of the virus (in $l \cdot TTSD_{50}$/ml) and interferon (in units/ml) in the brain of the mice maintained at various temperatures and infected with strains Sof .. and Sof K of virus TE

Strain of virus	Day after infection	temperature in degrees: 4 — titre: virus	4 — titre: interferon	18 — titre: virus	18 — titre: interferon	35 — titre: virus	35 — titre: interferon
Sof	1st	2.0	0	1.5	0	3.5	0
	3rd	5.0	40	5.0	40	5.33	160
	5th	5.5	640	5.5	1280	5.5	1280
Sof ..	1st	1.5	0	1.0	0	1.67	0
	3rd	3.5	0	3.0	0	4.0	20
	5th	4.67	40	4.5	40	5.0	640
	7th	5.0	320	5.33	640	-	-

The results of the research on preventive application of interferon are presented in Table 3. On the 5th day, all mice died which had not received interferon beforehand. A significant quantity of animals had died by this time in the group of mice which received interferon in the brain. The disease developed later in the mice receiving interferon intravenously, especially twice, and several mice survived: after an intravenous inoculation of 2560 units of interferon, 5 mice survived while in the corresponding control, there were none. The largest number, 7 mice, survived with intra-abdominal inoculation of interferon. Probably, the effectiveness of the endogenetic interferon with the infection, caused by virus TE depends not only on the quantity of the inoculated preparation but also on the route of inoculation. So, interferon inoculated intra-abdominally showed a pronounced (87.5%) preventive effect with the peripheral route of inoculation of the virus.

It is interesting to study the circulation of the inoculated interferon. For this, the mice were inoculated intravenously with 1280 units of interferon. A group of mice were inoculated repeatedly through 2 hours with the same dose. At 5, 15, 30 and 60 min. after the 1st and 2nd inoculation of interferon, blood and a brain sample were taken from the

animals. Interferon was prepared according to the described method from the serum and brain. Interferon quickly disappeared from the serum: at 5 min. after inoculation of titre, it was 160 units/ml, at 15 min. it was 80 units/ml and at 30 min. interferon was not formed (Table 4). If the interferon is injected twice, it disappears from the blood stream with the same speed as with a simultaneous injection. Not once did we detect interferon in the brain.

Table 3

[illegible] ACTIVITY OF HOMOLOGIC INTERFERON IN MICE

Route of inoculation of the interferon	Dose of interferon (units)	Number of mice receiving interferon	Day after inoculation								Number of surviving mice
			4th	5th	6th	7th	8th	9th	10th	11th	
			number of mice that died								
Intravenously	1200	30			1	2	3	6	6	5	7
Intravenously	1200	30		1			7	10	6	2	4
Intravenously	1250	30				6	3	6	4	3	3
In brain	40	30		1	2		6	4	4	10	1
In brain[1]	70	30		2	3	1	6	6	6		3
Control		30	2	6	4	6	12	2	-	-	0

[1] The total dose of interferon after 2 injections.

Table 4

[illegible] OF INTERFERON IN THE [illegible] OF THE BLOOD [illegible] EXPERIMENTS WITH RABBITS

Number of injections of interferon	Research material	Period of examination after inoculation (in min.)			
		5	15	30	60
		Titre of interferon (in units/ml)			
1[1]	Blood	160	80	0	0
1	"	160	80	0	0
1	"	0	0	0	0
1	Brain	0	0	0	0
1	"	0	0	0	0
1	"	0	0	0	0

[1] Each injection is given intravenously in a dose of 1250 units/ml.

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The rapid disappearance of interferon from the serum with intravenous injection was also noted by other authors [12]; there are also indications that interferon is quickly extracted by the kidneys but only 2% of it in this way, and the remaining accumulation is fixed by the cells of various organs, with the result that during subsequent infection the condition maintains partial or complete resistance [8]. In regards to the preventive action of interferon with the infection of mice by the viurs of the Berdiki fields, there are high potential possibilities of even smaller doses of interferon in the cases of infection by small doses. In the cases of infection by virus TE, interferon probably does not provide the animals a reliable defense since exogenic and, evidently, endogenic interferon, synthesized in the blood of the wool, do not penetrate the blood, the cells of which are targets for virus TE. Interferon, penetrating the blood simultaneously with the reproduction of the virus there, is an already delayed reaction which can hardly influence the flow of the infection process.

Summary

Correlation between interferon production and multiplication of tick-borne encephalitis virus in mice in relation to the degree of pathogenicity of viruses, routes of inoculation and the temperature of maintenance of inoculated animals.

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